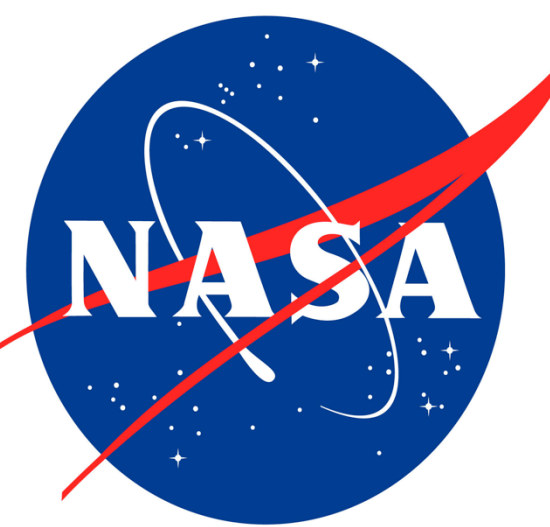


Effects of ionizing radiation on murine gene expression in skin and bone



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ABSTRACT

Long duration spaceflight causes a negative calcium balance and reduces bone density in astronauts. The potential for exposure to space radiation to contribute to lasting decrements in bone mass is not yet understood. Sustained changes to bone mass have a relatively long latency for development, however skin is a radiation sensitive organ and changes in skin gene expression may serve as an early radiation biomarker of exposures and may correlate with adverse effects on skeletal tissue. Previous studies have shown that FGF18 gene expression levels of hair follicles collected from astronauts on the ISS rose over time [1]. In the hair follicle, FGF18 signaling mediates radioresistance in the telogen by arresting the cell cycle, and FGF18 has the potential to function as a radioprotector [2]. In bone, FGF18 appears to regulate cell proliferation and differentiation positively during osteogenesis and negatively during chondrogenesis [3, 4]. Cellular defense responses to radiation are shared by a variety of organs, hence in this study, we examined whether radiation induced gene expression changes in skin may be predictive of the responses of skeletal tissue to radiation exposure. We have examined oxidative stress and growth arrest pathways in mouse skin and long bones by measuring gene expression levels via quantitative polymerase chain reaction (qPCR) after exposure to total body irradiation (TBI). To investigate the effects of irradiation on gene expression, we used skin and femora (cortical shaft) from the following treatment groups: control (normally loaded, sham-irradiated), and TBI (0.5 Gy ⁵⁶Fe 600 MeV/n and 0.5 Gy ¹H 150 MeV/n). Animals were euthanized one and 11 days post-IR. Statistical analysis was performed via a Student's t-test. In skin samples one day after IR, skin expression of FGF18 was significantly greater (3.8X) than sham-irradiated controls (3.8X), but did not differ 11 days post TBI. Expression levels of other radiation related genes (Nfe2l2, Trp53, Cdkn1a, FoxO3, Gadd45g, SOD1), was not different due to TBI at either time point. In bone (femora) TBI significantly increased (3.8X) expression of the pro-bone resorption cytokine, MCP-1, one day after TBI. FGF18 expression in skin and MCP-1 expression in bone were found to be positively correlated (P<0.002, r=0.8779). Further, microcomputed tomography analysis of tibiae from these animals showed reduced fractional cancellous bone volume (-21.7%) at 11 days post exposure. These results suggest that early radiation induced changes in FGF18 gene expression in skin may have value for predicting subsequent loss of cancellous bone mass.

INTRODUCTION

Background:

- Simulated space radiation induces bone loss.
- Skin is one of the most radiation-responsive organs.

Previous research:

We performed gene expression analysis on hair follicles from astronauts. FGF18 gene expression levels of hair follicles collected from astronauts on the ISS increased over time [1]. Exposure to gamma radiation (2Gy) increased expression of MCP-1 in pooled tibial and femoral marrow at 1 day post-irradiation [5].

Advantages of using hair follicles and skin:

Hair follicles and skin are relatively easy to acquire from subjects. Bone on the other hand, requires special imaging devices to be analyzed for structure and sampling for biochemical analyses is invasive.

Purpose of this study:

Determine whether skin can be used to predict the responses of bone to simulated microgravity and radiation

Long-term goal:

Develop a relatively simple diagnostic tool for bone loss by analyzing skin

Hypothesis:

Changes in skin gene expression may serve as an early radiation biomarker of radiation exposure and may correlate with adverse effects on skeletal tissue.

METHODS

Animal:

Male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME), 16 weeks of age

Experimental group:

- Control (Cont)
 - Radiation exposure (Rad) – 1 day and 11 days after total body irradiation
- Mice were exposed to a single dose of radiation consisting of 1 Gy of total body irradiation (0.5 Gy ⁵⁶Fe 600 MeV/n and 0.5 Gy ¹H 150 MeV/n) at a dose rate of 5 cGy/min (⁵⁶Fe) , 3 cGy/min (¹H) at the NASA Space Radiation Laboratory beamline at Brookhaven National Laboratory (BNL).

Extraction of RNA:

Total RNA was extracted from skin and femur (flushed of marrow) using Trizol.

Gene expression analysis:

Quantitative polymerase chain reaction (qPCR) was performed for the following genes:

Cdkn1a, FoxO3, SOD1, Gadd45g, Trp53, FGF18, Nfe2l2 (For skin). MCP-1, Nfe2l2, Rankl (For femur). Values are normalized to expression levels of L19. [n=5/group]

Microcomputed tomography (MicroCT):

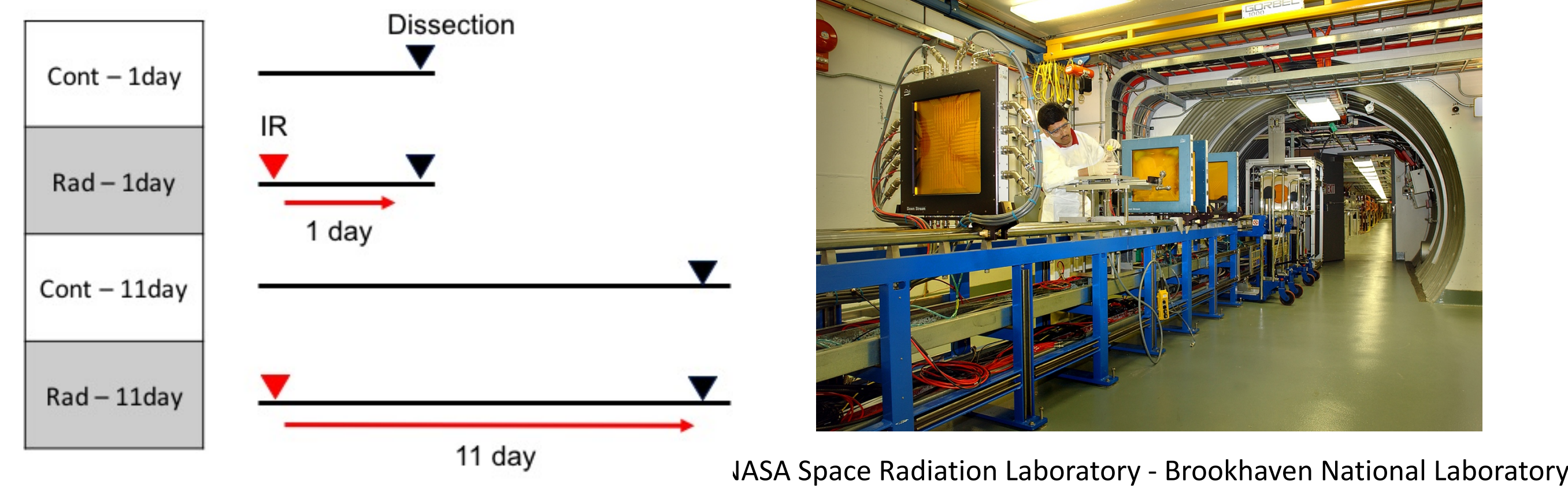
Tibiae were scanned by microCT. Bone volume per total volume (BV/TV) was calculated. [n=6/group]

Immunohistochemistry of skin:

The expression of FGF18 was analyzed using monoclonal antibodies specific for FGF18. The avidin-biotin immunohistochemical procedure was used for the localization of primary antibody binding according to manufacturer's instructions (ABC kit, Santa Cruz Biotechnology). [n=5/group]

Statistics:

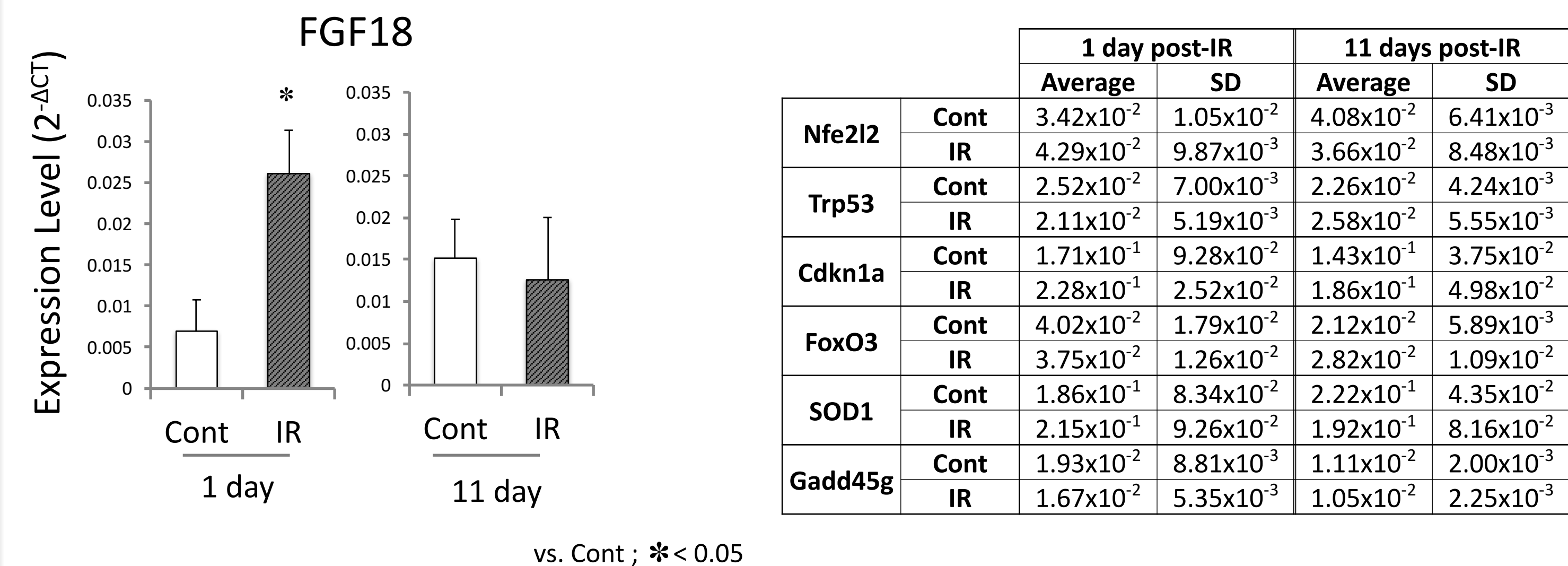
Data shown are means +S.D. Student T-test was performed, and P<0.05 accepted as significant.



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PILOT RESULTS

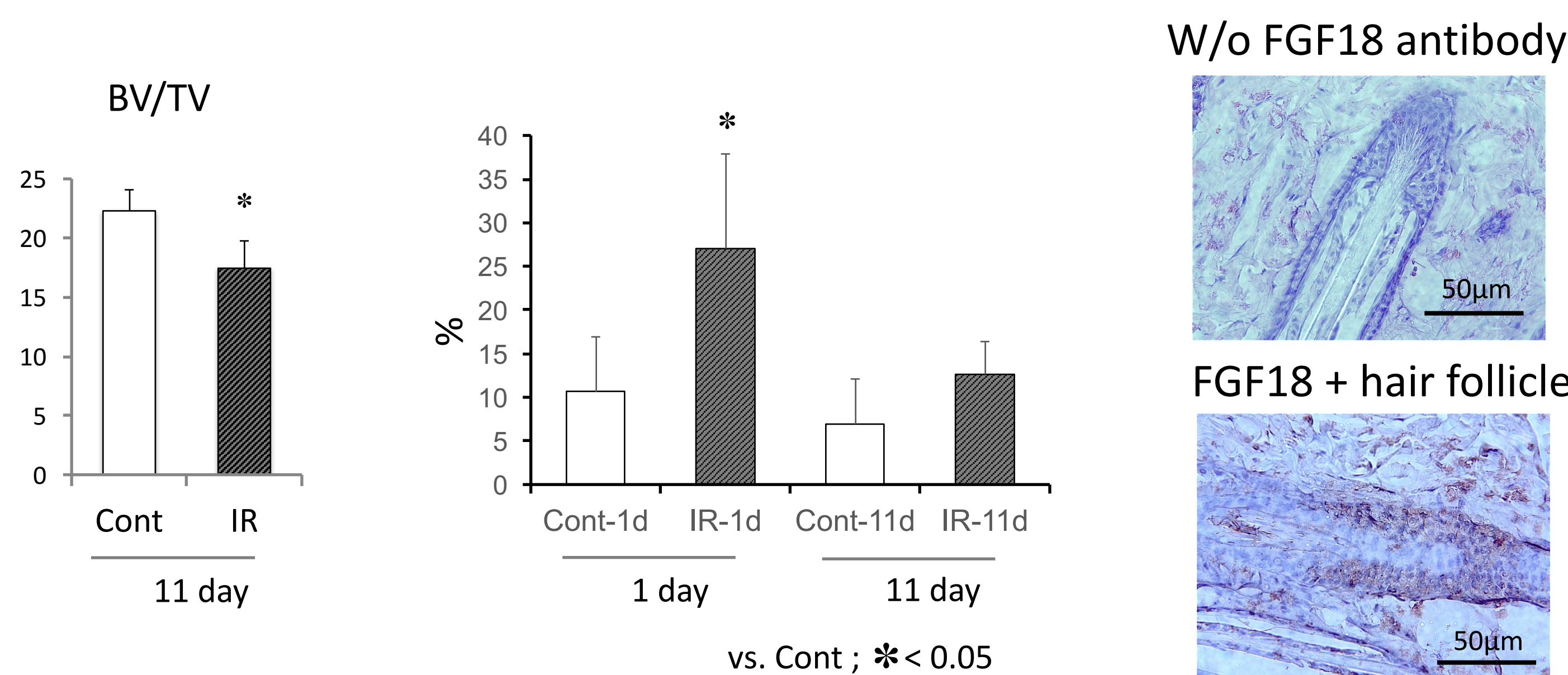
Gene expression in skin



In skin, FGF18 transiently increased 24 hours after irradiation (IR). This gene returned to the control level within 11 days post IR. No effects on gene expression were detected 1 day or 11 days post-IR.

Bone volume per total volume

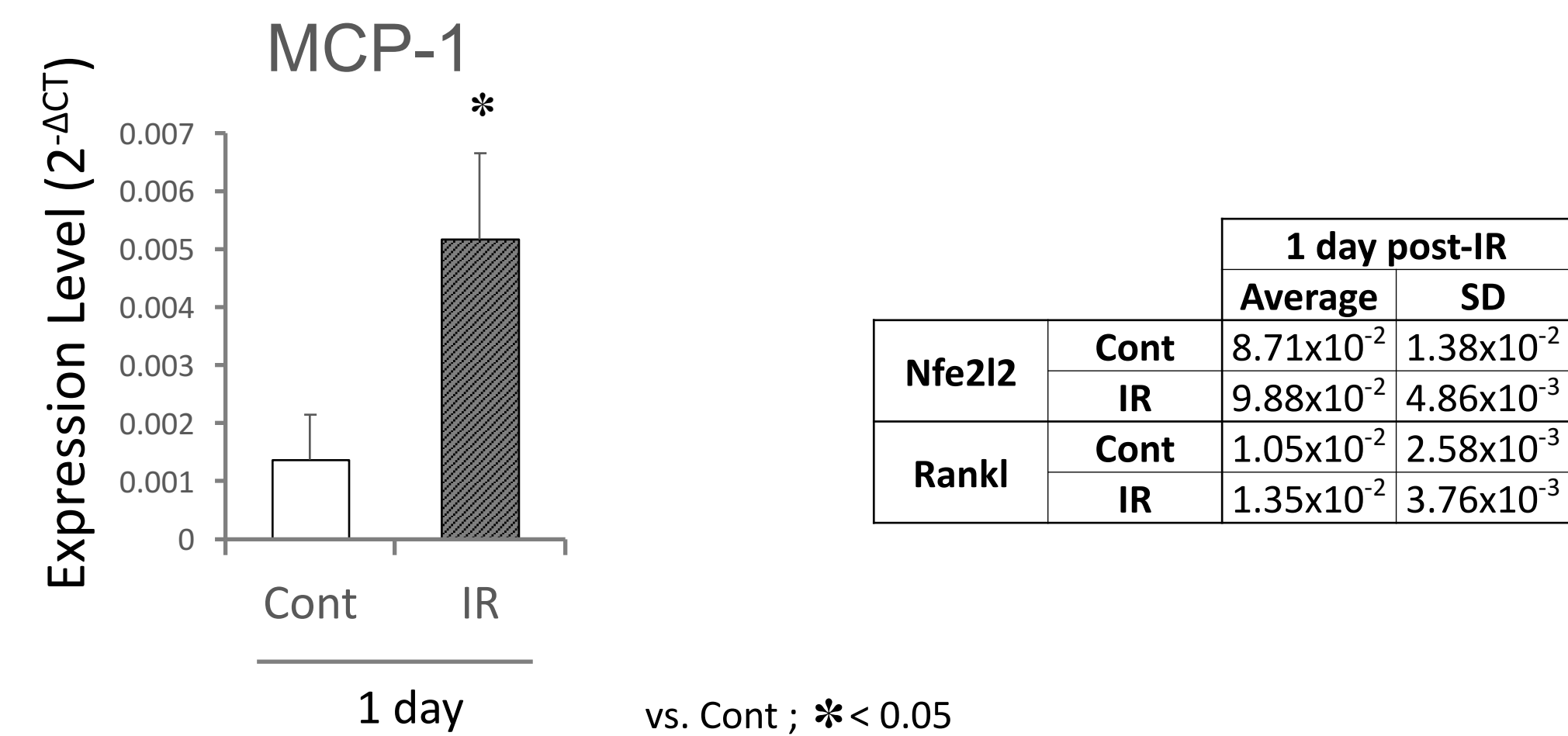
Percentage of FGF18+ hair follicles



IR significantly reduced BV/TV at 11 days post-IR.

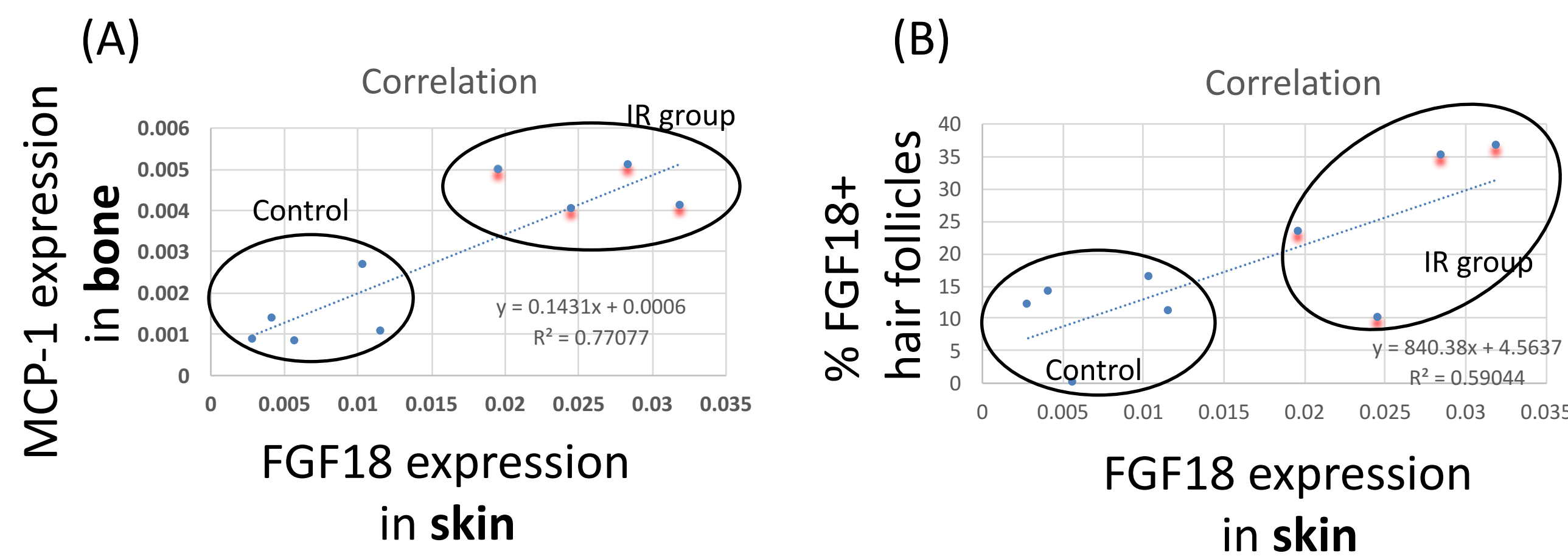
The percentage of FGF18+ hair follicles increased at one day post-IR and returned to basal levels at 11 days post-IR. The changes in gene expression and protein product of FGF18 occurred early after IR.

Gene expression in bone (femur)



In bone, expression of MCP-1 increased 24 hours after IR.

Correlation plot



		1 day post-IR	
		Correlation coefficient (r)	p value
FGF18 (skin)	MCP1 (bone)	8.78x10 ⁻¹	1.90x10 ⁻³
FGF18 RNA (skin)	% FGF18+ hair follicles	7.68x10 ⁻¹	1.56x10 ⁻²

Correlation of gene expression between skin and bone. (A) Correlation between skin FGF18 expression and bone MCP-1 expression. (B) Correlation between skin FGF18 expression and % FGF18+ hair follicles.

CONCLUSIONS

FGF18 expression in skin and MCP-1 expression in bone were strongly correlated 1 day after exposure to radiation (P<0.002, r=0.8779). Further, microcomputed tomography analysis of tibiae from animals at a later time (11 days) showed reduced cancellous bone volume/total volume (-21.7%) at 11 days post-IR. These results suggest that measurements of early radiation induced changes in FGF18 gene expression in skin may have value for predicting subsequent loss of cancellous bone mass. Further research may lead to the development of a relatively simple diagnostic tool for bone loss, with the advantage that hair follicles and skin are relatively easy to acquire from human subjects.

ACKNOWLEDGEMENTS

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